Vector Competence of Mexican and Honduran Mosquitoes (Diptera: Culicidae) for Enzootic (IE) and Epizootic (IC) Strains of Venezuelan Equine Encephalomyelitis Virus

MICHAEL J. TURELL,[1] MONICA L. O'GUINN, ROBERTO NAVARRO,[2] GUADELOUPE ROMERO,[3] AND JOSÉ G. ESTRADA-FRANCO[4]

Virology Division, U.S. Army Medical Research Institute of Infectious Diseases, 1425 Porter Street, Fort Detrick, Maryland 21702-5011

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ABSTRACT Experimental studies evaluated the vector competence of *Ochlerotatus taeniorhynchus* (Wiedemann), *Culex cancer* Theobald, *Culex pseudes* (Dyar and Knab), *Culex taeniopus* Dyar and Knab, and a *Culex* (*Culex*) species, probably *Culex quinquefasciatus* Say, and *Culex nigripalpus* Theobald from Chiapas, Mexico, and Tocoa, Honduras, for epizootic (IC) and enzootic (IE) strains of Venezuelan equine encephalomyelitis (VEE) virus. *Culex pseudes* was highly susceptible to infection with both the IC and IE strains of VEE (infection rates >78%). Patterns of susceptibility to VEE were similar for *Oc. taeniorhynchus* collected in Mexico and Honduras. Although *Oc. taeniorhynchus* was highly susceptible to the epizootic IC strains (infection rates ≥95%, $n = 190$), this species was less susceptible to the enzootic IE strain (infection rates ≤35%, $n = 233$). The *Culex* (*Culex*) species were refractory to both subtypes of VEE, and none of 166 contained evidence of a disseminated infection. Virus-exposed *Cx. pseudes* that refed on susceptible hamsters readily transmitted virus, confirming that this species was an efficient vector of VEE. Although *Oc. taeniorhynchus* that fed on hamsters infected with the epizootic IC strain transmitted VEE efficiently, only one of six of those with a disseminated infection with the enzootic IE virus that fed on hamsters transmitted virus by bite. These data indicate that *Cx. pseudes* is an efficient laboratory vector of both epizootic and enzootic strains of VEE and that *Oc. taeniorhynchus* could be an important vector of epizootic subtypes of VEE.

KEY WORDS *Ochlerotatus taeniorhynchus*, *Culex pseudes*, Venezuelan equine encephalomyelitis virus, transmission, Honduras, Mexico

VENEZUELAN EQUINE encephalomyelitis (VEE) virus is responsible for sporadic epizootics of severe disease, primarily in Central America and northern South America. Infection with epizootic subtypes of VEE often is fatal in horses and results in low mortality, but high morbidity in humans (Walton and Grayson 1989). Epizootics have extended from Peru in South America to as far north as Texas. A 1995 epidemic in Colombia and Venezuela (Weaver et al. 1996, Rivas et al. 1997) resulted in 75,000 to 100,000 human cases with at least 300 fatalities. Outbreaks of disease in horses in Chiapas, Mexico, in 1993 and in Oaxaca, Mexico, in 1996 were because of VEE subtype IE virus (Oberste et al. 1998). The reemergence of epidemic and enzootic VEE associated with equine mortality has increased interest in understanding the epidemiology and identifying potential vectors of both enzootic and epizootic strains of this virus.

Although VEE has been isolated from >40 species of mosquitoes (Sudia and Newhouse 1975, Walton and Grayson 1989), field isolation and laboratory vector competence studies indicate that different mosquito species may be responsible for transmission of epizootic and enzootic strains of VEE. Mosquito species implicated as potential vectors of epizootic IAB stains of VEE include *Psorophora columbiae* (Dyar and Knab), *Psorophora discolor* (Coquillett), *Ochlerotatus sollicitans* (Walker), *Ochlerotatus taeniorhynchus* (Wiedemann), *Mansonia indubitans* (Dyar and Shannon), and *Culex* (*Deinocerites*) *pseudes* (Dyar and Knab) (Sellers et al. 1965, Grayson and Galindo 1972, Sudia and Newhouse 1975, Walton and Grayson 1989). *Oc. taeniorhynchus* is an efficient laboratory vector of

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In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International.

[1] E-mail: michael.turell@det.amedd.army.mil.

[2] Animal Health Division, Agricultural Ministry, Chiapas, Mexico.

[3] Director of Epidemiology, Honduras Ministry of Health, Tegucigalpa, Honduras.

[4] Center for Agricultural Biotechnology, University of Maryland, College Park, MD 20742.

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14. ABSTRACT

Experimental studies were undertaken to evaluate the vector competence of Ochlerotatus taeniorynchus (Wiedemann), Culex cancer Theobald, Culex pseudes (Dyar and Knab), Culex taeniopus Dyar and Knab, and a Culex (Culex) species, probably Culex quinquefasciatus Say and Culex nigripalpus Theobald] from Chiapas, Mexico, and Tocoa, Honduras, for epizootic (IC) and enzootic (IE) strains of Venezuelan equine encephalitis (VEE) virus. Culex pseudes was highly susceptible to infection with both the IC and IE strains of VEE virus (infection rates > 78%). Patterns of susceptibility to VEE were similar for the Oc. taeniorynchus collected in Mexico and Honduras. Although Oc. taeniorynchus was highly susceptible to the epizootic IC strain (infection rates ≥ 95%, n = 191), this species was less susceptible to the enzootic IE strain (infection rates ≤ 30%, n = 311). The Culex (Culex) species were refractory to both subtypes of VEE virus, and none of 166 contained evidence of a disseminated infection. Virus-exposed Cx. pseudes that refed on susceptible hamsters readily transmitted virus, confirming that this species was an efficient vector of VEE virus. Although Oc. taeniorynchus that fed on hamsters infected with the epizootic IC strain transmitted VEE virus efficiently, only one of six of those with a disseminated infection with the enzootic IE virus that fed on hamsters transmitted virus by bite. These data indicate that Cx. pseudes is an efficient laboratory vector of both epizootic and enzootic strains of VEE virus and that Oc. taeniorynchus could be an important vector of epizootic subtypes of VEE.

15. SUBJECT TERMS
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Table 1. Susceptibility of mosquitoes collected near Chiapas, Mexico to enzootic, subtype IE stains of Venezuelan equine encephalomyelitis virus after feeding on hamsters with viremias of $10^{8.3 \pm 0.7}$ PFU/ml of blood

Species	Virus								
	68U201			93-42124			MX-01-22		
	n	Inf. (%)[a]	Dis. (%)[b]	n	Inf. (%)[a]	Dis. (%)[b]	n	Inf. (%)[a]	Dis. (%)[b]
Ochlerotatus taeniorhynchus	122	35	9	50	30	6	32	34	3
Culex (Dei.) pseudes	26	77	73	1	100	100		not tested	
Culex (Cul.) spp.[c]	57	0	0	34	0	0	25	0	0
Culex (Mel.) taeniopus	12	92	58		not tested			not tested	

[a] Percentage of mosquitoes containing virus.
[b] Percentage of mosquitoes containing virus in their legs.
[c] Probably *Cx. quinquefasciatus* and *Cx. nigripalpus*.

epizootic IAB and IC strains and an inefficient one of enzootic IE strains of VEE (Kramer and Scherer 1976, Turell 1999). In contrast, *Culex (Melanoconion) taeniopus* Dyar and Knab is highly susceptible to enzootic VEE subtype IE virus, but is nearly refractory to epizootic VEE subtypes IAB and IC viruses (Scherer et al. 1982, 1986, 1987, Turell et al. 1999). A variety of *Culex (Melanoconion)* species have been incriminated as potential vectors of enzootic ID and IE strains of VEE (Walton and Grayson 1989, Turell et al. 2000).

To determine potential vectors of VEE in Central America, mosquitoes were collected in Chiapas State, Mexico, and in Colon Department, Honduras, transported to a biological safety level-3 (BSL3) laboratory at the United States Army Medical Research Institute of Infectious Diseases (USAMRIID), and evaluated for their ability to become infected with and transmit epizootic and enzootic strains of VEE.

Materials and Methods

Mosquitoes. Adult female mosquitoes were collected by CDC miniature light traps (John W. Hock Co., Gainesville, FL) baited with dry ice or aspirated as they came to feed on horses near Pampa Honda, Mapastepec, Chiapas, Mexico (15° 20′ N, 93° 03′ W), in September 1999 and in April and May 2000, and from Las Coaches, Chiapas, Mexico, in November 2001. These mosquitoes were collected in the same general area as the 1993 VEE outbreak in Chiapas State. Mosquitoes also were collected near Tocoa and Trujillo, Colon Department, Honduras (15° 45′ N, 86° 00′ W) in dry ice-baited miniature light traps during August 2001. Mosquitoes were transported to a BSL3 laboratory at the USAMRIID (with HEPA-filtered exhaust air, treated sewage, and a 100% clothing change), provided apple slices as a carbohydrate source, and held at 26°C for 1–7 d until exposed to VEE virus. Species evaluated included *Oc. taeniorhynchus*, *Cx. (Deinocerites) pseudes*, *Culex (Deinocerites) cancer* Theobald, *Cx. (Melanoconion) taeniopus*, and *Culex (Culex)* species. Based on progeny rearings from some of the *Culex* specimens, these probably consisted of *Culex quinquefasciatus* Say and *Culex nigripalpus* Theobald. Voucher specimens were deposited at the National Museum of Natural History, Smithsonian Institution, Washington, DC.

Virus and Virus Assay. We used two epizootic IC strains of VEE, p676 (isolated from a mosquito captured during a VEE outbreak in Venezuela in 1963) and Col 95-1289 (isolated during an outbreak of VEE in Colombia in 1995). In addition, we used three enzootic IE strains: the 68U201 strain isolated from a sentinel hamster in Guatemala in 1968 (Scherer et al. 1970), the 93-42124 strain isolated from a dead horse in Mapastepec, Chiapas, Mexico (Oberste 1998), and the MX01-22 strain isolated from a sentinel hamster in Las Coaches, Chiapas, Mexico in July 2001. Although the 68U201and the p676 strains had received multiple cell culture passages, the Col 95-1289, 93-42124, and MX01-22 strains had received ≤2 cell culture passages before use in these studies. Virus titers were determined by plaque assay on Vero cell monolayers of serial 10-fold dilutions of specimens as described by Gargan et al. (1983), except that the neutral red stain was added 2 d after the initial plaque assay.

Determination of Vector Competence. Adult female Syrian hamsters were inoculated intraperitoneally with 0.2 ml of a suspension containing $\approx 10^4$ plaque-forming units (PFU) of one of the strains of VEE. These hamsters were anesthetized one or 2 d later and placed individually (i.e., one per cage) on the top of cages containing 50-150 unsorted, field-collected mosquitoes, or the F_1 progeny of these mosquitoes. Immediately after mosquito feeding, 0.2 ml of blood was obtained from each hamster by cardiac puncture and added to 1.8 ml of diluent (10% fetal bovine serum in medium 199 with Earle's salts and antibiotics). The blood suspensions were frozen at −70°C until assayed on Vero cell monolayers to determine the viremias at the time of mosquito feeding. After exposure to the viremic hamsters, engorged mosquitoes were transferred to 3.8-liter screen-topped cardboard cages. Apple slices or a 7% sucrose solution were provided as a carbohydrate source, and mosquitoes were held at 26°C and a photoperiod of 16:8 (L:D) h for 14 or 15 d. To determine if the mosquitoes could transmit virus by bite, mosquitoes were allowed to feed on susceptible hamsters either individually or in small groups of 2–5 mosquitoes each. Because VEE infection consistently is fatal to hamsters, we considered death of these animals to indicate virus transmission. Presence of virus was verified by isolating virus from brain tissue from a subset of the

Table 2. Potential of mosquitoes collected near Chiapas, Mexico, to transmit Venezuelan equine encephalomyelitis virus after feeding on hamsters with viremias of $10^{8.3 \pm 0.7}$ PFU/ml of blood

Species	Virus							
	IE				IC			
	n	Inf. (%)[a]	Dis. (%)[b]	Est. trans. rate[c]	n	Inf. (%)[a]	Dis. (%)[b]	Est. trans. rate[c]
Ochlerotatus taeniorhynchus	204	34	7	1	125	100	84	79
Culex (Dei.) pseudes	27	78	74	74	24	96	92	69
Culex (Cul.) spp.[d]	116	0	0	0	42	5	0	0
Culex (Mel.) taeniopus	12	92	58	58			not tested	

[a] Percentage of mosquitoes containing virus.
[b] Percentage of mosquitoes containing virus in their legs.
[c] Estimated transmission rate = percentage of that species that developed a disseminated infection after oral exposure × transmission rate for mosquitoes with a disseminated infection. Transmission rates for mosquitoes with a disseminated infection were 1/6 and 15/16 for *Oc. taeniorhynchus* with the IE and IC virus strains, respectively, and 3/3 and 3/4 for *Cx. pseudes* with the IE and IC virus strains, respectively.
[d] Probably *Cx. quinquefasciatus* and *Cx. nigripalpus*.

dead hamsters. Immediately after each transmission trial, mosquitoes were killed by freezing at −20°C for 5 min, identified to species, and their legs and bodies triturated separately in 1 ml of diluent. These suspensions then were frozen at −70°C until tested for virus.

Mosquito infection was determined by recovering virus from its body tissue suspension. If virus was recovered from its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. In contrast, if virus was recovered from both body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). The dissemination rate was the percentage of orally exposed mosquitoes that contained virus in their legs. Because mosquitoes were tested for transmission in small pools, it was not always possible to determine which mosquito in a pool actually transmitted virus by bite. Therefore, if more than one mosquito with a disseminated infection fed in a pool, data from that pool were not used to calculate the transmission rate, regardless of hamster survival.

Results and Discussion

Mean viremias in hamsters infected with the IC and IE strains of VEE were $10^{8.5}$ (range, $10^{8.3}$ to $10^{8.9}$) and $10^{8.3}$ (range, $10^{7.5}$ to $10^{9.0}$) plaque forming units (PFU)/ml during mosquito feedings, respectively. The viremias to which mosquitoes were exposed in our study were comparable to those observed in donkeys (Mackenzie et al. 1976) and in horses (Justines et al. 1981) inoculated with epizootic IC strains, and in bats (Seymour et al. 1978) inoculated with an enzootic IE strain of VEE virus. Infection and dissemination rates for mosquitoes from Mexico that fed on hamsters infected with each of the three strains of subtype IE virus were similar ($\chi^2 \leq 0.54$, df ≤ 2, $P \geq 0.46$ or Fisher exact test, $P > 0.74$) (Table 1). Therefore, data for the three strains were combined (Table 2). Likewise, data for mosquitoes that fed on hamsters infected with either of the two strains of subtype IC virus were nearly identical and these data also were combined (Table 2).

Culex pseudes was highly susceptible to infection with both the IC and IE subtypes of VEE (infection rates ≥78%), and nearly all infected individuals developed a disseminated infection by 14 d after ingestion of either the IC or IE subtypes of VEE. There was no significant difference in either infection or dissemination rates by subtype of virus ingested (Fisher exact test, $P \geq 0.10$) (Table 2). Not only were the *Cx. pseudes* highly susceptible to both the IC and IE strains of VEE virus, but also six of seven individuals with a disseminated infection that took a second blood meal transmitted virus by bite (Table 3). Therefore, this species was an efficient laboratory vector of both epizootic and enzootic strains of VEE. Similarly, the *Cx. cancer* collected in Honduras (Table 4) and the *Cx. taeniopus* collected in Mexico (Table 2) were highly susceptible to infection after ingesting VEE subtype IE virus, although the dissemination rate for *Cx. cancer* was lower ($\chi^2 = 11.9$, df = 1, $P < 0.001$) than that observed with *Cx. pseudes*. Neither species was tested with the IC sub type. The results with *Cx. taeniopus* were similar to those reported by Scherer et al. (1982, 1986) and Turell et al. (1999). Although *Cx. taeniopus* is highly susceptible to enzootic VEE subtype IE virus, it is a less efficient vector of epizootic VEE subtypes IAB and IC viruses (Scherer et al. 1982, 1986, 1987, Turell et al. 1999). Therefore, despite its ability to efficiently transmit the enzootic IE subtype of VEE, *Cx. taeniopus* probably would not be an important vector of epizootic IAB or IC subtypes. However, because of their ability to efficiently transmit the IE subtype of VEE and because both species feed on horses (Martin et al. 1973, Cupp et al. 1986), both *Cx. pseudes* and *Cx. taeniopus* might have been important vectors during outbreaks in Chiapas and Oaxaca.

Table 3. Potential for mosquitoes from Chiapas, Mexico, with a disseminated infection to transmit Venezuelan equine encephalomyelitis virus

Species	Virus			
	IE		IC	
	n fed	trans. rate[a]	n fed	trans. rate[a]
Ochlerotatus taeniorhynchus	6	17	16	94
Culex (Dei.) pseudes	3	100	4	75
Culex (Mel.) taeniopus	2	100	not tested	

[a] Percentage of mosquitoes that transmitted virus.

Table 4. Susceptibility of mosquitoes collected near Tocoa and Trujillo, Honduras, to infection with Venezuelan equine encephalomyelitis virus after feeding on hamsters with viremias of $10^{8.3 \pm 0.3}$ PFU/ml of blood

Species	Virus					
	IE (93-42124)			IC (Col 95-1289)		
	n	Inf. (%)[a]	Dis. (%)[b]	n	Inf. (%)[a]	Dis. (%)[b]
Ochlerotatus taeniorhynchus	29	14	0	65	95	75
Culex (Deinocerites) cancer	12	92	8		not tested	
Culex (Culex) spp.[c]	4	0	0	4	0	0

[a] Percentage of mosquitoes containing virus.
[b] Percentage of mosquitoes containing virus in their legs.
[c] Probably *Cx. quinquefasciatus* and *Cx. nigripalpus*.

In contrast to the other species tested, the *Culex* (*Culex*) species were refractory to both strains of VEE, and none of 158 contained evidence of a disseminated infection. This lack of susceptibility of the *Culex* (*Culex*) is consistent with earlier studies (Kissling and Chamberlain 1967, Schaffer and Scherer 1974, Kramer and Scherer 1976, Turell 1999, Turell et al. 2000) that also found that *Culex* (*Culex*) species were generally refractory to both epizootic and enzootic strains of VEE.

For both epizootic and enzootic subtypes of VEE, infection and dissemination rates for *Oc. taeniorhynchus* from Mexico and Honduras were similar ($\chi^2 \leq 2.63$, df = 1, $P \geq 0.11$) (Tables 2 and 4). Therefore, the data for this species were combined for further analysis. Although *Oc. taeniorhynchus* was highly susceptible to the epizootic IC strains (infection rate = 98%, $n = 191$), significantly ($\chi^2 = 230$, df = 1, $P < 0.001$) fewer (29%, $n = 311$) became infected when they ingested the enzootic IE strain (Tables 2 and 4). The disparity between the IC and the IE viruses was even greater when comparing disseminated infection rates; 84% developed a disseminated infection after feeding on the IC subtypes which was significantly ($\chi^2 = 297$, df = 1, $P < 0.001$) greater compared with only 8% of those that fed on one of the IE subtypes. In addition, although 94% (15 of 16) *Oc. taeniorhynchus* with a disseminated VEE IC infection transmitted virus by bite, only 17% (1 of 6) with a disseminated VEE IE infection transmitted virus (Table 3). This difference also was significant (Fisher exact test, $P = 0.001$). A moderate salivary gland barrier was detected in a North American strain of *Oc. taeniorhynchus* tested with the 68U201 strain of VEE, because only nine (47%) of 19 VEE virus-inoculated mosquitoes transmitted this virus by bite (M.J.T., unpublished data). Therefore, not only was this species less susceptible to VEE IE virus via the oral route, but they also had a salivary gland barrier to the IE, but not the IC strains of VEE. This was consistent with an earlier study by Kramer and Scherer (1976). The high susceptibility to the IC strain of VEE was consistent with an earlier study by Turell (1999) with *Oc. taeniorhynchus* collected in Venezuela. A recent study by Brault et al. (2002) found that the susceptibility of *Oc. taeniorhynchus* to enzootic and epizootic strains of VEE was because of a molecular determinant on the E2 envelope glycoprotein and may explain why this species is an efficient vector of epizootic strains, yet an inefficient transmitter of enzootic strains. Therefore, *Oc. taeniorhynchus* is not likely to be involved in the maintenance of enzootic subtypes of this virus. However, because of efficient laboratory vector competence for the epizootic IAB and IC strains, large populations that may occur with this species, long flight range, preference for feeding on large mammals (Cupp et al. 1986), and high viremias in horses infected with epizootic subtypes of VEE virus (Justines et al. 1981), *Oc. taeniorhynchus* may be important in the rapid spread of epizootic strains of VEE virus.

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References Cited

Brault, A. C., A. M. Powers, and S. C. Weaver. 2002. Vector infection determinants of Venezuelan equine encephalitis virus reside within the E2 envelope glycoprotein. J. Virol. 76: 6387–6392.

Cupp, E. W., W. F. Scherer, J. B. Lok, R. J. Brenner, G. M. Dziem, and J. Ordonez. 1986. Entomological studies at an enzootic Venezuelan equine encephalitis virus focus in Guatemala, 1977–1980. Am. J. Trop. Med. Hyg. 35: 851–859.

Gargan, T. P., II, C. L. Bailey, G. A. Higbee, A. Gad, and S. El Said. 1983. The effect of laboratory colonization on the vector pathogen interactions of Egyptian *Culex pipi-*

ens and Rift Valley fever virus. Am. J. Trop. Med. Hyg. 32: 1154–1163.

Grayson, M. A., and P. Galindo. 1972. Experimental transmission of Venezuelan equine encephalitis virus by *Deinocerites pseudes* Dyar and Knab, 1909. J. Med. Entomol. 20: 196–200.

Justines, G., G. Oro, and O. Alvarez. 1981. Venezuelan equine encephalitis virus: horse virulence of P-676 and MF-8 small and minute plaques. Am. J. Trop. Med. Hyg. 30: 444–448.

Kissling, R. E., and R. W. Chamberlain. 1967. Venezuelan equine encephalitis virus, pp. 65–84. *In* Advances in Veterinary Science, vol. II, Academic, New York

Kramer, L. D., and W. F. Scherer. 1976. Vector competence of mosquitoes as a marker to distinguish Central American and Mexican epizootic from enzootic strains of Venezuelan encephalitis virus. Am. J. Trop. Med. Hyg. 25: 336–346.

Mackenzie, R. M., J. de Siger, and D. Parra. 1976. Venezuelan equine encephalitis virus: comparison of infectivity and virulence of strains V-38 and P676 in donkeys. Am. J. Trop. Med. Hyg. 25: 494–499.

Martin, D. H., B. N. Chaniotis, and R. B. Tesh. 1973. Host preferences of *Deinocerites pseudes* Dyar & Knab. J. Med. Entomol. 25: 206–208.

Oberste, M. S., M. Fraire, R. Navarro, et al. 1998. Association of Venezuelan equine encephalitis virus subtype IE with two equine epizootics in Mexico. Am. J. Trop. Med. Hyg. 59: 100–107.

Rivas, F, L. A. Diaz, V. M. Cardenas, et al. 1997. Epidemic Venezuelan equine encephalitis in La Guajira, Colombia, 1995. J. Infect. Dis. 175: 828–832.

Schaffer, P. A., and W. F. Scherer. 1974. Growth of Venezuelan equine encephalitis virus and disappearance of Coxsackie A, Rous sarcoma, herpes simplex and vaccinia viruses following inoculation of *Aedes aegypti* and other mosquitoes. J. Med. Entomol. 11: 189–196.

Scherer, W. F., R. W. Dickerman, and J. V. Ordonez. 1970. Discovery and geographical distribution of Venezuelan encephalitis virus in Guatemala, Honduras, and Br. Honduras during 1965–1968, and its possible movement to Central Am. and Mexico. Am. J. Trop. Med. Hyg. 19: 703–711.

Scherer, W. F., E. W. Cupp, G. M. Dziem, R. J. Brenner, and J. V. Ordonez. 1982. Mesenteronal infection threshold of an epizootic strain of Venezuelan equine encephalitis virus in *Culex (Melanoconion) taeniopus* mosquitoes and its implication to the apparent disappearance of this virus strain from an enzootic habitat in Guatemala. J. Med. Entomol. 31: 1030–1037.

Scherer, W. F., S. C. Weaver, C. A. Taylor, and E. W. Cupp. 1986. Vector incompetency: its implication in the disappearance of epizootic Venezuelan equine encephalomyelitis virus from Middle America. J. Med. Entomol. 23: 23–29.

Scherer, W. F., S. C. Weaver, C. A. Taylor, and E. W. Cupp, R. W. Dickerman, and H. H. Rubino. 1987. Vector competence of *Culex (Melanoconion) taeniopus* for allopatric and epizootic Venezuelan equine encephalomyelitis viruses. Am. J. Trop. Med. Hyg. 36: 194–197.

Seymour, C., R. W. Dickerman, and M. S. Martin. 1978. Venezuelan encephalitis virus infection in neotropical bats. II. Experimental infections. Am. J. Trop. Med. Hyg. 27: 297–306.

Sellers, R. F., G. H. Bergold, O. M. Suarez, and A. Morales. 1965. Investigations during Venezuelan equine encephalitis outbreaks in Venezuela—1962–1964. Am. J. Trop. Med. Hyg. 14: 460–469.

Sudia, W. D., and V. F. Newhouse. 1975. Epidemic Venezuelan equine encephalitis in North America: a summary of vector-host relationships. Am. J. Epidemiol. 101: 1–13.

Turell, M. J. 1999. vector competence of three Venezuelan mosquitoes (Diptera: Culicidae) for an epizootic IC strain of Venezuelan equine encephalitis virus. J. Med. Entomol. 36: 407–409.

Turell, M. J., T. P. Gargan, I. I., and C. L. Bailey. 1984. Replication and dissemination of Rift Valley fever virus in *Culex pipiens*. Am. J. Trop. Med. Hyg. 33: 176–181.

Turell, M. J., J. Barth, and R. E. Coleman. 1999. Potential For Central American mosquitoes to transmit epizootic and enzootic strains of Venezuelan equine encephalitis virus. J. Am. Mosq. Control Assoc. 15: 295–298.

Turell, M. J., J. W. Jones, M. R. Sardelis, et al. 2000. Vector Competence of Peruvian mosquitoes (Diptera: Culicidae) for epizootic and enzootic strains of Venezuelan equine encephalomyelitis virus. J. Med. Entomol. 37: 835–839.

Walton, T. E., and M. A. Grayson. 1989. Venezuelan equine encephalomyelitis, pp. 203–231. *In* T. P. Monath [ed.], The Arboviruses: Epidemiology and Ecology, vol. 4. CRC, Boca Raton, FL.

Weaver, S. C., R. Salas, R. Rico-Hesse, et al. 1996. Re-emergence of epidemic Venezuelan equine encephalomyelitis in South America. Lancet. 348: 436–440.

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